J Forensic Sci, September 2007, Vol. 52, No. 5
doi: 10.1111/j.1556-4029.2007.00530.x
Available online at: www.blackwell-synergy.com

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Age Estimation by Pulp/Tooth Ratio in Canines by Mesial and Vestibular Peri-Apical X-Rays

ABSTRACT: Changes in the size of the pulp canal, caused by apposition of secondary dentine, are the best morphometric parameters for estimating age by X-rays. The apposition of secondary dentine is the most frequently used method for age estimation in adult subjects. In two previous papers, we studied the application of the pulp/tooth area ratio by peri-apical X-rays as an indicator of age at death. The aim of the present study was to test the accuracy of age evaluation by combined analysis of labio-lingual and mesial peri-apical X-rays of lower and upper canines. A total of 200 such X-rays were assembled from 57 male and 43 female skeletons of Caucasian origin, aged between 20 and 79 years. For each skeleton, dental maturity was evaluated by measuring the pulp/tooth area ratio according to labio-lingual and mesial X-rays on upper (x_1, x_2) and lower (x_3, x_4) canines. Very good agreement was found between intra-observer measurements. Statistical analysis showed that all variables x_1, x_2, x_3, and x_4 and the first-order interaction between x_1 and x_3 contributed significantly to the fit, so that they were included in the regression model, yielding the following regression formula:

$$\text{Age} = 120.737 - 337.112x_1 - 79.709x_2 - 364.534x_3 - 65.655x_4 + 1531.918x_1x_3$$

The residual standard error of estimated ages was 3.62 years, with 94 degrees of freedom, and the median of the residuals was −0.155 years, with an interquartile range of 4.96 years. The accuracy of the method was ME = 2.8 years, where ME is the mean prediction error. The model also explained 94% of total variance (R^2 = 0.94).

KEYWORDS: forensic science, age determination, forensic odontology, dental pulp, pulp/tooth area, stepwise linear regression

Several age estimation methods exploit the various types of changes undergone by teeth, including wear (1–3), root dentine transparency (4–6), and apposition of secondary dentine (7–11). Dental pulp is a mesenchymal tissue surrounded by a pulp canal. Outside the pulp are some odontoblast lines, which release dentine during the subject's life and reduce the size of the pulp canal (Fig. 1). Changes in its size caused by the apposition of secondary dentine are the best morphometric parameters for estimating age by X-rays.

The apposition of secondary dentine is one of the methods most often used for age estimation in adult subjects. It is a continuing, regular process, which is only modified by caries or particular abrasion. Secondary dentine has been studied by several methods, both sectioning and X-rays. One method used by us for age estimation involved orthopantomographs (OPG) of the maxillary canines, to study the pulp/tooth area ratio (11). Another method used peri-apical X-rays of canines (12). Both the methods reduce possible errors caused by magnification and distortion of X-rays and provide better image quality.

Canines were chosen for a number of reasons: they are normally the oldest teeth, undergo less wear as a result of diet than posterior teeth, are less likely than other anterior teeth to suffer wear as a result of particular work, and are the single-root teeth with the largest pulp area and thus the easiest to analyze. Age estimation by apposition of secondary dentine (7–11) only studies modifications visible in labio-lingual X-rays. However, some important information may also be obtained by mesial X-rays. Volumetric study by computerized tomography (CT) (13) would be the best method, but it is normally impossible to apply. Simultaneous study of mesial and labio-lingual sides is important for more complete results.

The aim of this study was to test the accuracy of age evaluation by combined analysis of labio-lingual and mesial peri-apical X-rays of lower and upper canines.

Materials and Methods

Peri-apical X-rays of 200 canines from 57 male and 43 female skeletons, with known ages at death of between 20 and 79 years, were analyzed (Table 1). The teeth were taken from the osteological collection of Sassari (Sardinia, Italy), preserved in the Museum of Anthropology, Department of Experimental and Evolutionistic Biology, University of Bologna, Bologna, Italy. The sample used is that evaluated in a previous paper (11), and is made up of 114 canines of males and 86 of females. Subjects' ages ranged between 20 and 77 years for males, and 20 and 79 years for females. Canines without pathologies were chosen. Peri-apical X-rays in the labio-lingual and mesial positions were taken digitally (FARO Production) at an exposure of 10 ma, 70 Kvp. Following Cameriere et al. (10), the radiographic images of the canines were processed by a computer-aided drafting program (AutoCAD2000, Install Shield 3.0, 1997) (It is also possible to use other drafting programs, such as ADOBE Photoshop). As reported by Paewinsky et al. (10), 20 points from each tooth outline and 10 points for each

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Received 22 Oct. 2006; and in revised form 16 Feb. 2007; and 3 April 2007; accepted 15 April 2007; published 6 Aug. 2007.

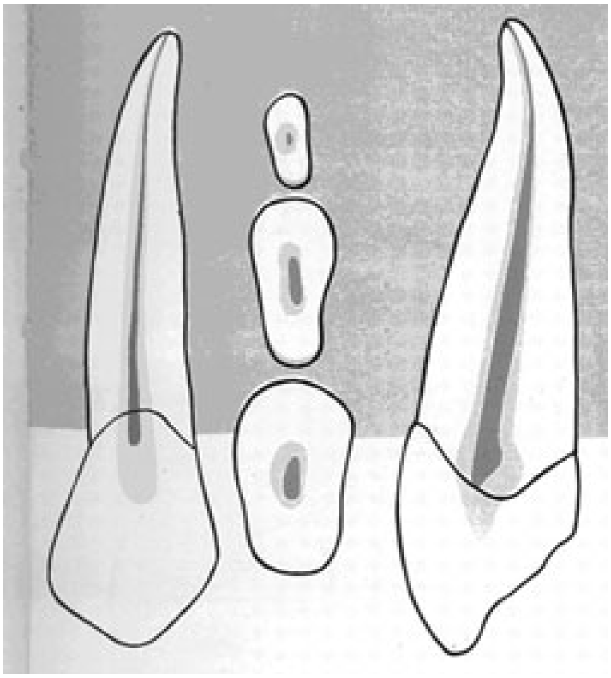

FIG. 1—*Pulp canal reduction with age.*

TABLE 1—*Age and sex distribution of study sample.*

Age (years)	Male	Female
20–29	11	8
30–39	14	9
40–49	12	10
50–59	13	9
60–69	5	2
>70	2	5
Total	57	43

pulp outline were identified and used to evaluate both tooth and pulp areas (mm^2). The ratio between pulp and tooth areas was used as a morphological variable.

Statistical Analysis

For each skeleton, dental maturity was evaluated by measuring the pulp/tooth area ratio on upper labio-lingual (x_1) and mesial (x_2), lower labio-lingual (x_3) and mesial (x_4) X-ray images of canines. All measurements were carried out by the same observer. To test intra-observer reproducibility, a random sample of 20 peri-apical X-rays was re-examined after an interval of 2 weeks. Intra-observer reproducibility of measurements was studied using the concordance correlation coefficient. The four morphological variables, x_1, x_2, x_3, and x_4, were entered in an EXCEL file for use as predictive variables for age estimation in the subsequent statistical analysis. Actual age at death was also recorded. Correlation coefficients were evaluated between age and predictive variables. To obtain an estimate of age at death as a function of the variables, a multiple linear regression model with first-order interactions was developed by selecting those variables that contributed significantly to age estimation using the stepwise selection method. Besides evaluating the age using measurements of canines from both jaws, we also evaluated separate predictions, restricted exclusively to either maxillary or mandibular canines. Statistical analysis was performed with S-PLUS 6 statistical programs (S-PLUS 6.1 for Windows, Professional Edition, Release 1). The significant threshold was set at 5%.

In addition, to evaluate the accuracy of the regression model, subjects' ages at death ($\mathrm{Age}_i, i = 1, \ldots, n;$ with $n = 100$) were compared with estimated ones ($\mathrm{Age}_{\mathrm{est}i}, i = 1, \ldots, n;$ with $n = 100$) using the mean prediction error (ME):

$$\mathrm{ME} = \frac{1}{n}\sum_{i=1}^{n} E_i = \frac{1}{n}\sum_{i=1}^{n} |\mathrm{Age}_i - \mathrm{Age}_{\mathrm{est}i}|,$$

where each $E_i, (i = 1, \ldots, n)$ is the absolute value of the difference between the age of the ith individuals.

Finally, to validate the regression model, a new sample of 10 skeletons was chosen at random, and peri-apical X-rays of their canines were analyzed. Then, the ages at death of these skeletons were compared with estimated ages according to the regression equation.

Results

There were no statistically significant intra-observer differences between the paired sets of measurements carried out on the re-examined peri-apical radiographs ($p < 0.01$). Pearson's correlation coefficients between age and morphological variables showed that all of them were significantly correlated with age, and that all correlation coefficients between age and morphological variables were significant and negative. Age at death was modeled as a function of the morphological variables (predictors) and, to optimize the model, a stepwise regression procedure was applied. A summary of the results is listed in Table 2. Statistical analysis showed that all the variables x_1, x_2, x_3 and x_4 and the first-order interaction between x_1 and x_3 contributed significantly to the fit (Table 2), so that they were included in the regression model, yielding the following regression formula:

$$\begin{aligned}\mathrm{Age} = {} & 120.737 - 337.112x_1 - 79.709x_2 - 364.534x_3 \\ & - 65.655x_4 + 1531.918x_1x_3 \qquad (1)\end{aligned}$$

This model had the lowest Akaike Information Criterion (AIC) value among the considered multiple regression models, as there was a modest improvement in model fit when the other

TABLE 2—*Stepwise regression analysis predicting chronological age from chosen predictors.*

	Value	SE	*t*-value	*p*-value
Upper and lower canines				
Intercept	120.737	4.495	26.86	<0.001
x_1	−337.112	56.884	−5.926	<0.001
x_2	−79.709	31.297	−2.547	0.012
x_3	−364.534	53.873	−6.766	<0.001
x_4	−65.655	28.893	−2.272	0.025
x_1 x_3	1531.918	435.7	3.516	0.001
Upper canine				
Intercept	111.75	2.9	38.54	<0.001
x_1	−373.78	33.62	−11.12	<0.001
x_2	−182.92	32.17	−5.69	<0.001
Lower canine				
Intercept	102.09	2.4	42.5	<0.001
x_3	−318.25	25.68	−12.39	<0.001
x_4	−177.89	25.58	−6.95	<0.001

SE, standard error.
x_1 x_3 specifies interaction between variables x_1 and x_3

first-order interaction between the morphological variables or terms of three or greater were included in the model. The residual standard error of the estimated ages was 3.62 years, with 94 degrees of freedom, and the median of the residuals was −0.155 years, with an interquartile range (IQR) of 4.96 years. The accuracy of the method was ME = 2.8 years. Model (1) also explained 94% of total variance ($R^2 = 0.94$).

The residual plot (Fig. 2, left) shows no obvious pattern, and the observed versus predicted plot (Fig. 2, right) shows that the regression model fits the trend of the data reasonably well. Hence, both diagnostic plots support our chosen model.

Separate parameter estimates for upper and lower canines are also given in Table 2.

When only the upper canine was considered, the regression equation was:

$$\text{Age} = 111.75 - 373.78x_1 - 182.92x_2 \qquad (2)$$

When only the lower canine was included, the equation became:

$$\text{Age} = 102.09 - 318.25x_3 - 177.89x_4 \qquad (3)$$

The coefficient of determination for regressions (2) and (3) was $R^2 = 0.897$ and 0.909, respectively. Although these values were slightly weaker than the coefficient of determination when both upper and lower canines were included in the model, it still remained highly significant.

When predicted age was obtained using equation (2), the residual standard error was 4.74 years and the median of the residuals was 0.95 years, and the IQR = 7.26 years (Fig. 3). The accuracy of the method was ME = 3.89 years. Using equation (3), the residual

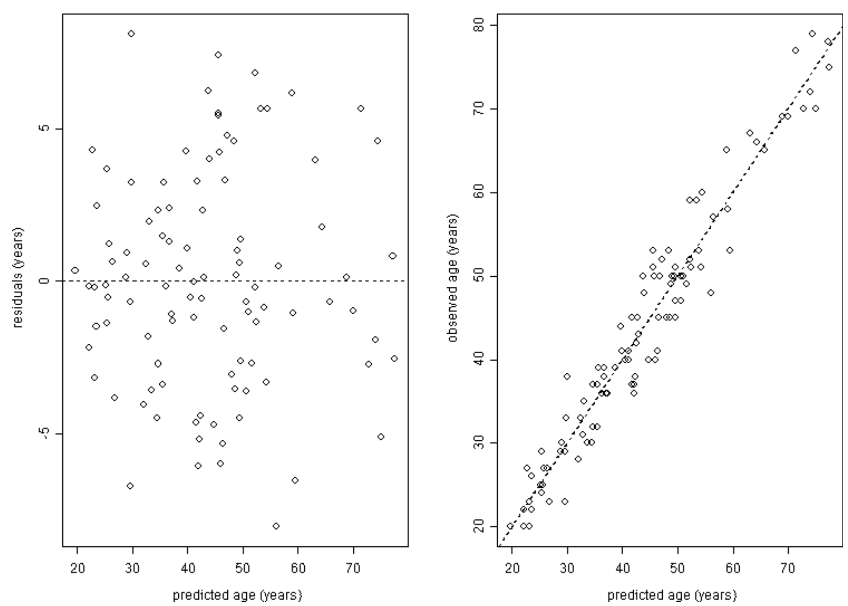

FIG. 2—*Plots of residuals against fitted values (left) and of observed against predicted values (right) using regression model (1).*

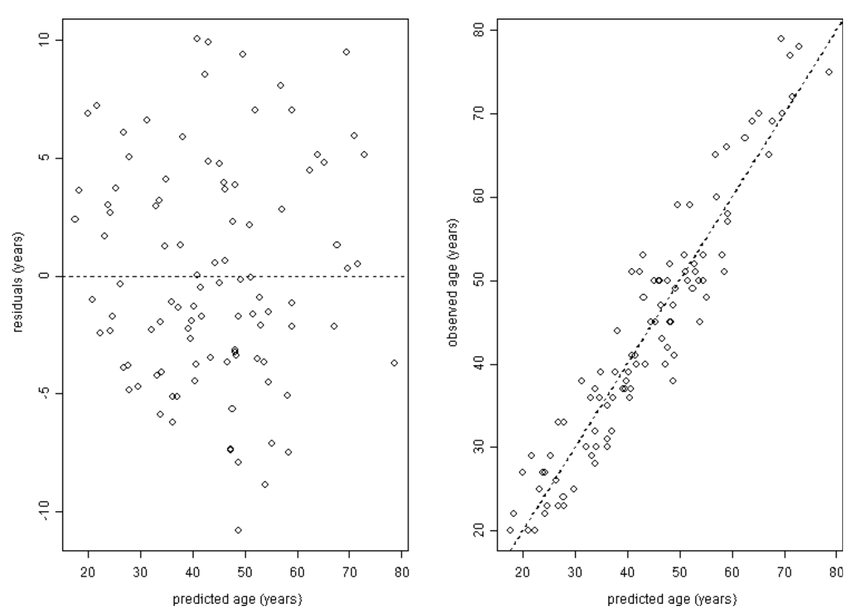

FIG. 3—*Plots of residuals against fitted values (left) and of observed against predicted values (right) using regression model (2).*

standard error was 4.47 years and the median of the residuals was 0.505 years, IQR = 5.93 years (Fig. 4). The accuracy of the method was ME = 3.59 years. The residual plots (Figs 3 and 4, left) show no obvious pattern. The observed versus predicted plots (Figs 3 and 4, right) show that the regression models fit the trend of the data reasonably well. Only a small increase in residual errors was seen when the simplest linear models (Eqs (2) and (3), with only upper or lower canines as independent variables) were used.

Discussion

Teeth are that part of the body most frequently employed for age estimation when skeletal remains are in poor condition, as humidity, fire or trauma, for example, may make many parts of the body unusable.

In previous studies, we evaluated the importance of the apposition of secondary dentine as an age indicator in adult subjects (10,11,14). The results, in line with other studies, have highlighted the correlation between age and apposition of secondary dentine (7–11,14). The radiographic techniques used to measure pulp changes is tried and tested and is non-destructive. All studies evaluate modifications observed in both labio-lingual and peri-apical X-rays and OPG. Peri-apical X-rays are fast, inexpensive, and routinely used in dentistry. The increasing use of digital X-rays has increased the quality of images and the capacity to discriminate the different parts of teeth.

These evaluations only allow bidimensional estimation. The best solution would be volumetric analysis but, as it requires CT, it is usually impossible to apply. However, a simple fast solution to improve results is to add the information from labio-lingual X-rays to those from lateral X-rays. The latter projection is possible only with extracted teeth, and this method is therefore only applicable to the skeletal remains. In both forensic and anthropological issues, most cases of age estimation in adults are made from skeletons.

In the method applied here, the choice to use canines for evaluation was particularly important, as lateral modifications are clearcut and easy to see. The size of the pulp area in canines, also in lateral X-rays, reveals modifications better than other teeth with smaller pulp areas (e.g., II incisor) or pluriradicular teeth (e.g., molars). The smaller size of the other single-root teeth leads to less clear measurement of the pulp/root ratio. In pluriradicular teeth, pulp changes are clear in the canal but less evident in the root. In addition, in adult subjects, molars and premolars are often missing or damaged as a result of wear.

Regression equation (1), using both canines as independent variables, showed higher R^2 and lower AIC and IQR than regression equations (2) and (3). Hence, the age-at-death estimates obtained using model (1) turned out to be better than those obtained using only one canine. The results obtained with single canines were similar to those obtained with labio-lingual X-rays. Instead, age-at-death estimates obtained with the pulp/tooth area ratio on both canines and both labio-lingual and mesial projections were significantly better than those evaluated by measuring the pulp/tooth area ratio on upper and lower canines only by labio-lingual X-rays.

In fact, as reported by Cameriere et al. (12), the age-at-death estimates obtained by measuring the pulp/tooth area ratio on upper and lower canines, by labio-lingual X-rays only, yielded a median of residuals of −0.772 years, IQR = 6.16 years, and ME = 3.36 years, whereas in the present study the values were −0.155 years, IQR = 4.96 years, and ME = 2.8 years, respectively.

These results indicate the appropriateness of using canines as morphological variables to predict individuals' ages, and the importance of including all possible information. Two different projections yield a larger amount of information and a more accurate age evaluation. Hence, if both canines, upper and lower, are available, the results are better using both projections, lateral and labio-lingual. The improvement in R^2 and the reduction of SD and ME make this technique the best for age estimation by canine X-rays. However, if only one canine is available, the small difference between the estimates according to labio-lingual X-rays, and both types of X-rays mean that only the labio-lingual projection is required. However, it is preferable to use both, although the improvement in accuracy is small, as taking one extra X-ray is simple.

These results allow us to evaluate age with good approximation both in forensic cases, when evaluations must be as precise as possible, and in anthropological cases, when the quality of the body

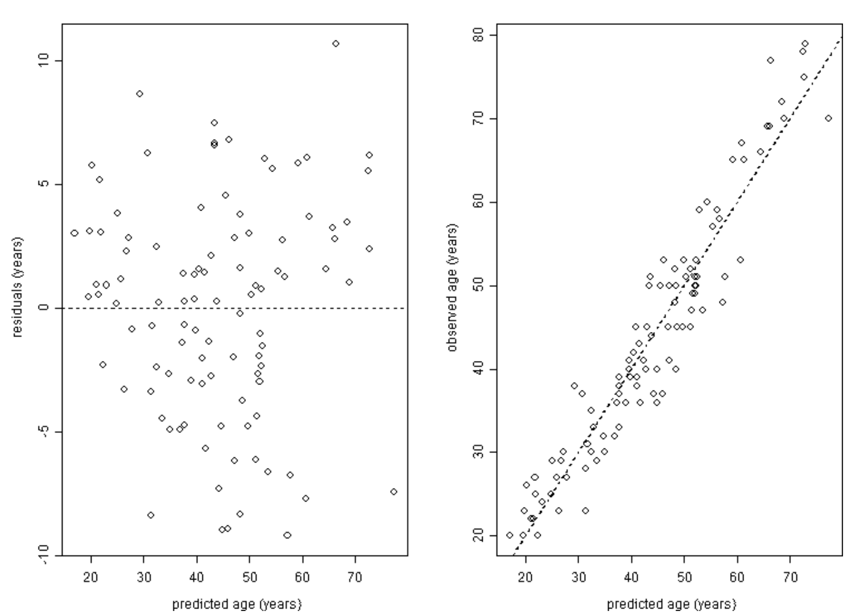

FIG. 4—*Plots of residuals against fitted values (left) and of observed against predicted values (right) using regression model (3).*

may be poor. As regards the influence of wear and diet on the reliability of our method, previous papers were examined which dealt with samples belonging to various periods [ancient peoples (14) and subjects from the 20th (12) and 21st centuries (11)]. The results showed the uniform reliability of the method and consequently indicated that the errors in age estimation using the apposition of secondary dentine is independent of historical period.

Future research should aim at acquiring data about other teeth, both single- and pluriradicular, and more in-depth study of the influence of wear, diet, and historical period on apposition of secondary dentine.

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